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An Encapsulated Dopamine-Releasing Polymer Alleviates Experimental Parkinsonism in Rats

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The effect of sustained intrastriatal release of dopamine (DA) from polymer matrices on apomorphine-induced turning behavior in a 6-hydroxydopamine (6-OHDA) unilaterally lesioned rat model was analyzed. A biocompatible semipermeable tube was placed in a denervated striatum as a receptacle for DA-releasing polymer rods. *In vitro* kinetics showed sustained release of DA from a polymeric rod for 15 days. Implantation of a DA-releasing rod within the striatal receptacle significantly decreased apomorphine-induced rotational behavior in lesioned animals. Upon removal of the DA-releasing system from the receptacle, rotational behavior increased within 2 weeks and approached preimplant control values 4 weeks later. Acute microdialysis revealed that DA appeared in the extracellular space within 20 min after the implantation of a DA-releasing rod into a denervated striatum. Significant DA amounts were still measurable 7 days postimplantation, indicating sustained DA release from the polymer rod. Dopamine released from a polymer matrix through a semipermeable receptacle alleviates experimental parkinsonism in rats, suggesting that controlled intrastriatal release of DA from a polymer matrix may provide an alternative method for the treatment of Parkinson's disease. © 1989 Academic Press, Inc.

INTRODUCTION

Parkinson's disease is characterized by a striatal dopamine deficiency secondary to the degeneration of the nigrostriatal pathway (10, 16). Transplantation of dopaminergic neurons has been reported to ameliorate the behavioral deficits in animals with experimentally induced parkinsonism (3, 22, 23). When embryonic mesencephalon was transplanted into a denervated striatum, extension of DA-containing processes was observed for long distances into the host brain (4, 9). The degree of behavioral recovery correlated with the extent of axonal outgrowth, suggesting that dopamine release by a diffuse neuritic network was responsible for the observed effect (4, 9). Behavioral recovery has also been reported with transplants of adrenal medulla (11). Even though fluorescence histochemical studies revealed the presence of dopamine in the transplant, the lack of extensive reinnervation of the host brain raises questions about the mechanism of behavioral recovery (12). Striatal sprouting from the host has been reported when adrenal medulla tissue was transplanted, suggesting a possible trophic influence provided by the transplanted tissue (6).

To separate diffusional effects from cell-mediated transport, transplantation of neural tissue in a polymer capsule has been attempted (1). It was reported that encapsulated mouse embryonic mesencephalon could survive for several weeks in rat brains (1) and partially corrected apomorphine-induced rotational behavior even in the absence of any synaptic contact between the transplanted tissue and the host brain (2). The molecular weight cutoff of the capsule membrane protects the transplanted tissue against immune rejection while allowing free diffusion of small molecules such as dopamine. To assess whether behavioral recovery observed with encapsulated transplants can be explained solely by diffusion of dopamine released by the transplant, a dopamine-releasing polymer rod was introduced in semipermeable polymeric capsules in unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats.

Utilizing polymer tubes previously shown to be biocompatible (1, 27), a device was designed to permit direct striatal access, preventing subsequent damage to target tissues during placement or retrieval of dopamine (DA)-releasing polymer matrices. To overcome DA autooxidation in solutions, a solid phase slow-release polymer system, similar to that of Langer *et al.* (13, 19, 20), was used to distribute crystalline DA particles in a hydrophobic matrix. Behavioral recovery under apomorphine challenge was assessed in a 6-OHDA unilaterally lesioned rat model. *In vivo* dopamine diffusion was measured by microdialysis.

MATERIALS AND METHODS

Animal model. Young adult (200–225 g) male Sprague–Dawley rats (Charles River Laboratory, Wilmington, MA) were anesthetized by intramuscular injec-

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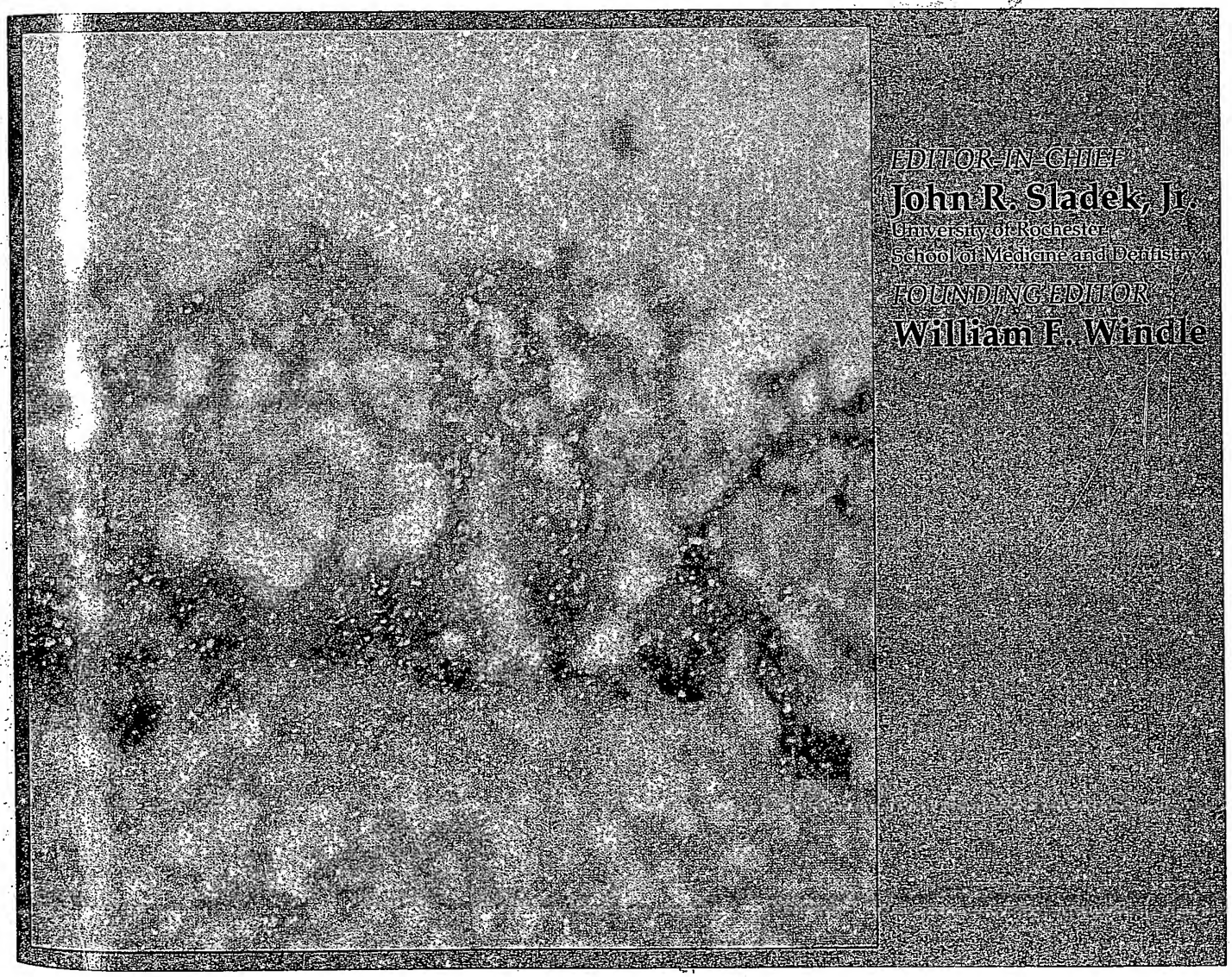

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tion of an 87/13 mg/kg mixture of ketamine (Ketalar)/xylazine (Rompun). Stereotaxic injections of 6-OHDA (12 μg 6-OHDA in 6 μl of 0.9% saline with 0.05 mg/ml of ascorbic acid) were performed into the anteriomedial region of the substantia nigra (coordinates: −2.9 mm bregma; 2.1 mm lateral, and 8.1 mm deep to the dura with the incisor bar set at 5.0 above the intraaural line). Two weeks after the lesion, rotational behavior was assessed under apomorphine (APO; 0.05 mg/kg sc) challenge. Behavior was characterized both in an open-field and modified Ungerstedt rotometer (25). Animals exhibiting more than eight turns per minute over a 40-min test period were selected for the study. Groups of three animals were housed in plastic cages on a 12-h on–off light cycle, with food and water available *ad libitum*.

Intracranial cannulation. Sixteen animals received intrastriatal receptacles made of a 9.5 mm long (0.85 mm in i.d.) semipermeable polyvinyl chloride acrylic copolymer (AC) tube fabricated by a phase inversion dry-jet wet spinning technique (7). The tubular membrane featured a molecular weight exclusion of 50 kilodaltons (kDa). The distal end of the tube was occluded with a solution of the same acrylic copolymer. The first 6 mm from the open end of the tube were coated with a polyurethane solution, rendering this portion impermeable, and therefore limiting fluid exchange to the striatum. Sterilized receptacles were inserted stereotaxically into the striatum (+0.3 mm bregma, 2.7 mm lateral to the midline, and 8.0 mm deep to the dura with the incisor bar set 3.0 below the intraaural line). Once implanted, the receptacles were secured by equidistant placement of two bone screws into the skull, providing anchorage for dental cement. The proximal port was closed with an AC glue. The receptacle remained *in vivo* for the duration of the study. Ten of these animals were used for behavioral studies, whereas the remaining 6 animals were used for *in vivo* microdialysis studies.

DA-releasing EVAc fabrication. Ethylene–vinyl acetate copolymer (EVAc) resin (40% by weight vinyl acetate, Elvax 40w, DuPont, Inc., Wilmington, DE) was washed 20 times in distilled water and 20 times in 95% ethanol to remove impurities. Purified EVAc was subsequently dissolved in methylene chloride to make a 10% (w/v) solution. Dopamine (DA) (Sigma, St. Louis, MO) was ground in a mortar to a fine powder and added to the EVAc solution to a final concentration of 20% DA to EVAc (w/w). The DA/EVAc solution was ultrasonicated for 5 min, agitated in a vortex mixer for 15 min, and rapidly cooled in liquid nitrogen in order to form a solid matrix with fixed DA particles. The methylene chloride was removed by lyophilization.

Strings with a diameter of 0.5 mm were pressure extruded at a temperature of 55°C and sectioned into 8-mm-long rods (26). To retard DA release, three coats of pure polymer were applied to each rod by repeated immersion in a 10% EVAc solution, resulting in a final diameter of 0.7 mm. The distribution of DA particles in the EVAc was analyzed by scanning electron microscopy (AMRay-1000A).

In vitro release kinetics. *In vitro* DA-release kinetics were studied by placing an 8-mm-long rod in 1 ml of 0.9% physiologic saline with 0.05 mg/ml of ascorbic acid incubated in individual wells at 37°C. At daily time points, the fluid was collected and its concentration was measured by HPLC with an electrochemical detector. The system used included a Model 5700 solvent delivery module and a model 5100A Coulochem multielectrode electrochemical detector (ESA, Bedford, MA). A 20-μl aliquot of each sample was injected onto the column (CA-HR 80; ESA) with no sample pretreatment. The mobile phase contained 0.05 *M* Na PO_4, 0.2 *M* EDTA, 212 mg/liter heptane sulfonic acid, and 3% methanol, at a pH of 2.6. Total run time was approximately 8 min. The concentration of each compound was determined by comparison with the peak height of serial-diluted standards run with each assay. The DA detection limit of the chromatographic system used was 50 pg.

The wells were replenished with fresh saline/ascorbate solution after each measurement. The DA release was calculated as cumulative percentage release.

In vivo implantation of EVAc/DA rods. Successfully lesioned animals with striatal receptacles were anesthetized and placed in a stereotaxic apparatus. Following midline incision, the proximal cap on the receptacle was located and excised, and a 20% DA/EVAc rod was loaded into the receptacle. The proximal end of the receptacle was again sealed with the AC glue. Skin closure was achieved with 6-0 monofilament nylon (Ethilon).

Rotation behavior was evaluated under apomorphine challenge (0.05 mg/kg) at 7 and 14 days post-DA/EVAc loading. The DA/EVAc rod was subsequently removed from the receptacle under methoxyflurane anesthesia at Day 14. Behavior was analyzed 2 and 4 weeks later.

Catecholamine determination by microdialysis. The microdialysis probes used in these experiments were composed of 50 kDa Mw cutoff AC semipermeable tubes (600 μm in i.d., 8 mm long) fabricated by a phase-inversion dry-jet wet spinning technique (7). A few hours prior to the experiment, the dialysis probe recovery was determined by placing the probe in a beaker of artificial cerebrospinal fluid (CSF) (150 mmol Na^+, 3.0 mmol K^+, 1.4 mmol Ca^{2+}, 0.8 mmol Mg^{2+}, 1.0 mmol PO_4, 155 mmol Cl^-, pH 7.4) containing known concentrations of DA and DHBA at 800 pg/20 μl (with 1 mg ascorbic acid/100 ml solution). DA concentration was determined by HPLC with an electrochemical detector (EC) as previously described. The relative recovery of the dialysate probes was 24–29% at room temperature. Dialysate val-

ues are reported as picograms per 20-min collection period.

For *in vivo* analyses, a dialysis probe was stereotaxically placed in proximity to the previously implanted receptacle in the rat striatum. The animal was anesthetized as previously described. Artificial CSF was pumped through the probe at a flow rate of 2.5 μl/min throughout the experiment. The dialysate was collected over 20-min intervals into tubes containing 5 μl 1.1 *N* perchloric acid. The sample was analyzed immediately by HPLC–EC.

After collecting a number of samples to determine baseline extracellular fluid (ECF) DA overflow, a 20% DA/EVAc rod was placed in the receptacle. Dialysis samples were collected for 20-min intervals postimplantation to determine if ECF DA levels were affected by the DA-releasing rod. DA levels were determined acutely following the implantation of the DA/EVAc rods in three animals and 7 days postimplantation in the three remaining animals.

Histology. Upon completion of the study, deeply anesthetized animals were perfused transcardially as previously described (27). The brain was removed and sections 25 μm thick were cut on a freezing sliding microtome (AO Reichert Model 976 C) and either picked up directly on glass slides coated with 3-aminopropyltriethoxysilane or immersed directly in Tris buffer. Selected sections were stained for Nissl substance with cresyl violet. Other sections were processed for immunocytochemical localization of tyrosine hydroxylase (TH) utilizing the avidin–biotin procedure. Brain sections were incubated 2 days at 4°C in primary antisera to TH (Eugene Tech, Allendale, NJ). Incubations in the secondary antisera and the avidin–biotin complex (Vectors Labs, Burlingame, CA) were carried out at room temperature and the peroxidase reaction was developed as described elsewhere (27). Mounted slides were analyzed with a Zeiss IM 35 interfaced with a morphometric system (CUE-2, Olympus Corp., Lake Success, NY).

RESULTS

In vitro release kinetics. *In vitro* DA release was determined by comparing cumulative release of eight 0.7 × 8-mm 20% DA/EVAc rods in an ascorbate/saline at 37°C over 15 days (Fig. 1). Sustained release was observed over time. Total DA content prior to the release studies amounted to 340 ± 25 μg/rod.

The 20% DA/EVAc rods were examined by scanning electron microscopy (SEM) prior to and 2 weeks after implantation. Cross-sectional SEM revealed an even distribution of DA particles suspended throughout the polymer matrix (Fig. 2A). Two weeks after incubation in ascorbate/saline solution or in a brain receptacle, the polymer rods showed disseminated pits and holes, indicative of DA particule dissolution (Fig. 2B).

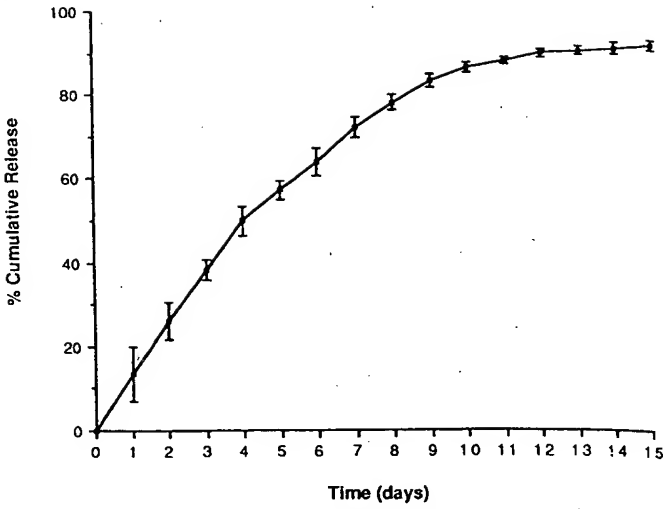

FIG. 1. Cumulative *in vitro* release kinetics of eight 0.7 × 8 mm 20% (w/w) DA/EVAc rods in 0.9% saline with 0.05 mg/ml of ascorbic acid at 37°C over 15 days. Bars represent means ± SD.

Rotational behavior. The implantation of empty striatal receptacles did not induce any additional neurological deficits in any of the animals. At the time of implantation of the EVAc rods, the proximal cap of the receptacle was hermetically fused to the tube. After cap removal, the receptacle lumen was filled with an acellular clear liquid. At the conclusion of the study, the location of the receptacle was determined by histological analysis, revealing consistent placement of the receptacle within the striatum (Fig. 3). Immunohistochemical localization of tyrosine hydroxylase on the substantia nigra and the striatum confirmed greater than 90% destruction of the nigrostratial pathway. No evidence of sprouting surrounding the receptacle was observed.

During the first few hours after implantation, the animals receiving DA/EVAc rods spontaneously rotated contralateral to the implant side, whereas animals receiving control implants did not exhibit such behavior. Figure 4 summarizes the effect of the APO challenge before, during, and after the implantation of a 20% DA/EVAc rods as compared to control animals, who had received implants of EVAc alone. Controls showed a slight improvement in rotational behavior 7 days postimplantation with return to preimplantation values at all subsequent time points. Experimental animals displayed a statistically significant decrease in rotational behavior as compared to controls at both 7 and 14 days postimplantation (46.0% at 7 days and 83.8% at 14 days). Two weeks after the removal of the DA-releasing rod, rotational behavior increased again, leaving no statistical difference between the control and experimental group at 4 weeks.

Microdialysis. Dopamine levels in the extracellular fluid of lesioned striata were consistently undetectable

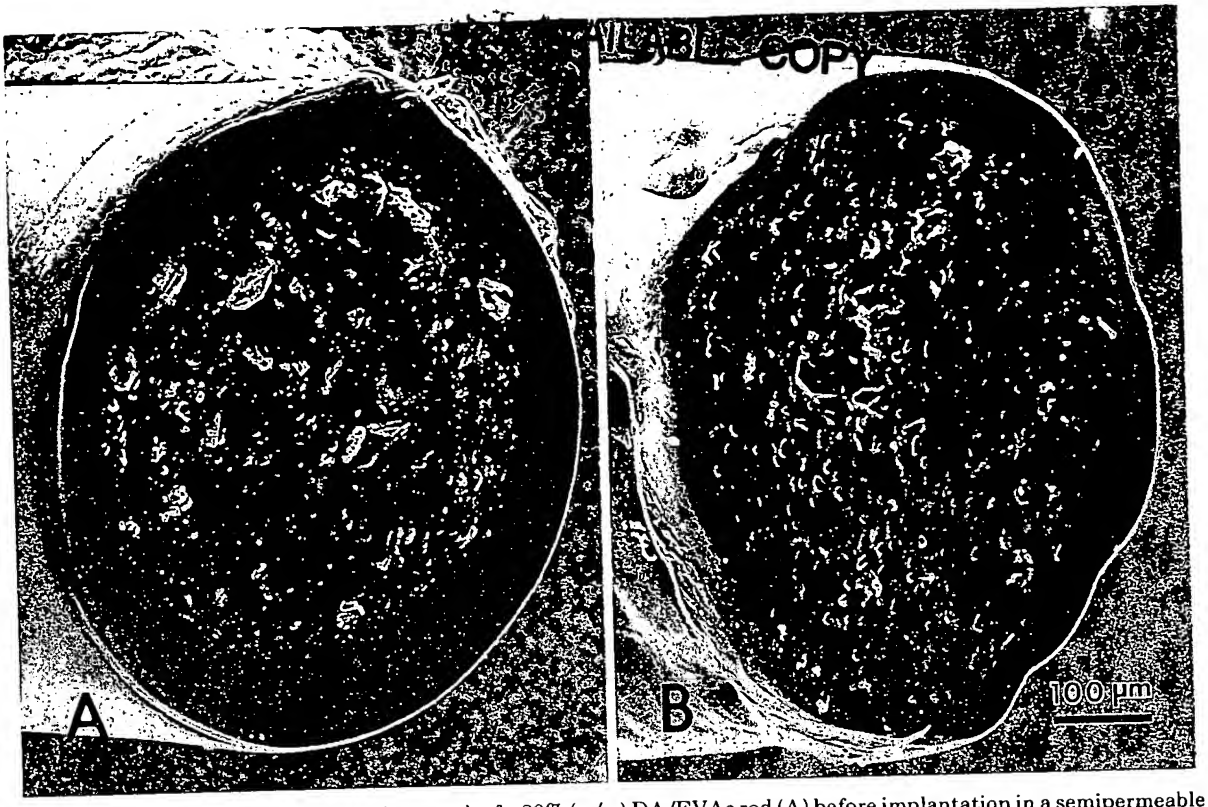

FIG. 2. Cross-sectional scanning electron micrograph of a 20% (w/w) DA/EVAc rod (A) before implantation in a semipermeable receptacle; the DA particles are distributed throughout the polymer matrix; note also the presence of pure EVAc at the periphery of the rod; (B) 14 days postimplantation in a brain receptacle. Note the disseminated pits and holes, indicative of DA particle dissolution.

by microdialysis. Twenty minutes after the implantation of a 20% DA-releasing EVAc polymeric rod, detectable levels of DA were recovered. The DA levels remained elevated throughout the next 180 min (Fig. 5). Significant extracellular striatal DA was present in the lesioned striatum 7 days postimplantation of DA/EVAc. Two animals had extracellular DA values in the 20 ng/20 µl range, whereas the third had values in the 200 ng

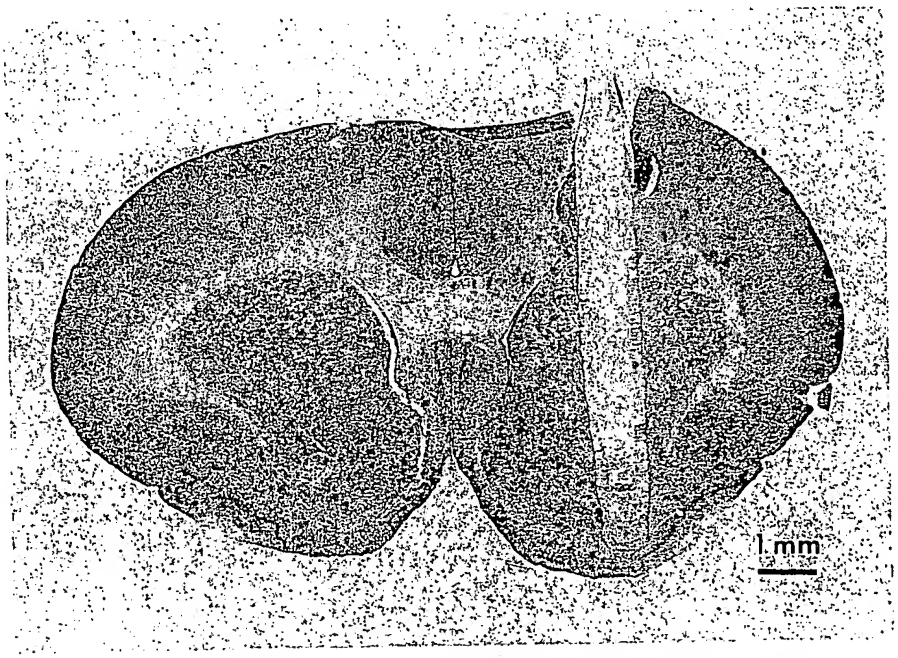

FIG. 3. Nissl-stained coronal section of a rat brain with a striatal receptacle 15 weeks postimplantation.

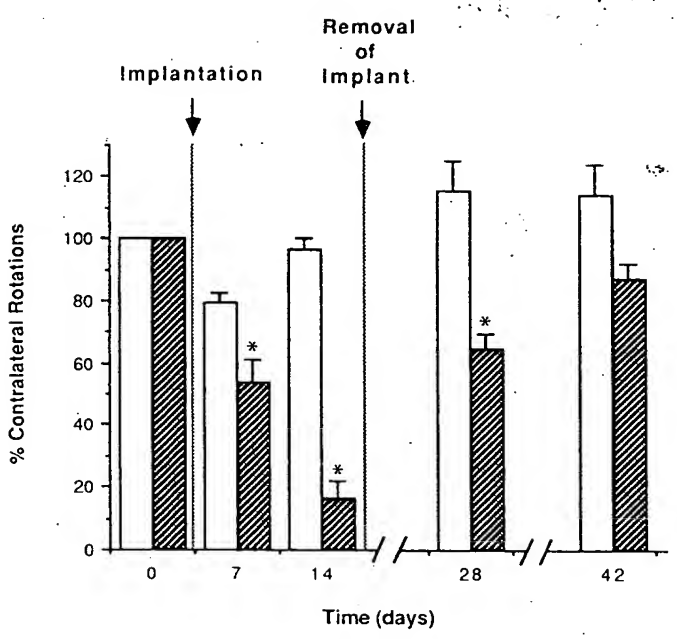

FIG. 4. Rotational behavior results of control ($n = 5$) versus experimental group ($n = 5$) as assessed with apomorphine challenge. As compared to the sham controls, the experimental animals showed a significant reduction in rotation behavior after the implantation of a dopamine containing rod with return to the preimplant value 28 days after removal of the implant. For each time point the experimental animals are compared to controls. *$P < 0.05$, Wilcoxon Mann–Whitney test (rank sum test). Bars represent means ± SEM. (□) control; (▨) experimental.

range. In all of these three animals the levels of DA remained constant over the duration of the experiments. Histologically, the microdialysis probes were found to be located 300–500 μm from the receptacles.

DISCUSSION

A limiting factor in determining the influence of direct striatal infusions of DA has been the relative instability of DA within aqueous reservoirs. *In vivo* autooxidation of DA and the limited potency and/or solubility of most available DA agonists have restricted progress of continuous *in vivo* infusions as a means to reduce motor deficits in Parkinson's disease (8).

The present study indicates that intrastriatal implantation of a sustained-release DA polymer within a receptacle provides a valuable tool to study denervated nigrostriatal functions. The *in vitro* kinetics of 20% (w/w) DA/EVAc rods showed that DA is continuously released over a period of 15 days. The *in vitro* release of dopamine should constitute a good predictor of its *in vivo* release, since the mechanism of drug release in EVAc copolymer relies on water penetration in the hydrophilic microchannels created by the crystalline DA particles. The present study reveals that sustained striatal DA release decreases rotational behavior deficits in unilaterally lesioned rats and that this effect is reversed after removal of the dopamine-releasing polymer. Microdialysis has revealed that DA release into the extracellular fluid is measurable within 20 min after the implantation of a DA-releasing polymer in a lesioned striatum. Seven days after the placement of the rod, significant levels of dopamine were present, suggesting sustained *in vivo* release. The variability observed in quantities of DA recovered from the ECF with microdialysis between the various animals may be multifactorial. These may include distances of probe placement from the receptacle, differences in the quantities of DA released from the EVAc rod, and to a lesser degree, the variability in a host's reuptake mechanism. If a minimum amount of DA is required to maintain a certain "tone," it is conceivable that the daily amount released in the present study could be greatly reduced and rods releasing drug more slowly for longer time periods should be fabricated.

Chronically infused dopamine or dopamine agonists from miniosmotic pumps also have been reported to alleviate experimental parkinsonism (8, 15, 24). However, solubility, stability, and reservoir limitations are drawbacks of this technology. Bioresorbable 1:1 polyglycolic and polylactic acid copolymer microcapsules containing dopamine particles have been utilized as another means to deliver dopamine (21). Controlled sustained release of a drug from a resorbable polymer relies on surface erosion. Polyesters are degraded mainly by hydrolysis, which entails bulk erosion rendering predictable release difficult. The use of a hydrophobic polymer such as eth-

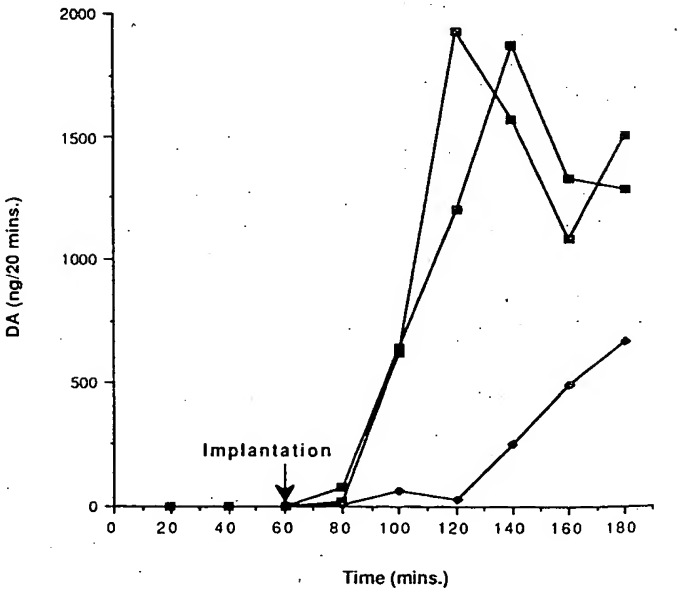

FIG. 5. Implantation of a 20% (w/w) DA-releasing EVAc rod into a receptacle in the lesioned striatum of three animals resulting in an elevation of extracellular DA to detectable levels within 20 min and continuing until the termination of microdialysis.

ylene–vinyl acetate copolymer offers the advantage of diminishing autooxidation while allowing more regular release rates.

An implantable intrastriatal receptacle provides a means for placement and removal of dopamine-releasing polymer rods. Therefore, animals can act as their own controls. If implantable releasing polymers are to become a realistic alternative to the current clinical treatment of Parkinson's disease, ways of removing and replacing a depleted implant under local anesthesia must be provided. Furthermore, a receptacle could be placed in any central nervous system structure for site-specific delivery of therapeutic agents. However, long-term *in vivo* biocompatibility studies must be undertaken in a primate model to ascertain potential complications such as establishment of a diffusion barrier or infection.

Björklund *et al.* have recently speculated on the possible mechanisms involved in the actions of intracerebral neural implants (5). They support the idea that improvements in behavioral deficits are multifactorial; i.e., trophic, neurohumoral, and synaptic mechanisms each have the capacity to improve host brain function. Polymer science technology provides tools for identifying the mechanisms involved in intracerebral grafting procedures, for instance the Mw cutoff of tubular membranes can be controlled so as to exclude certain factors, while permitting the passage of others. The present study shows that DA released from a polymer matrix, diffusing through a semipermeable membrane, and acting at the striatum ameliorates the behavioral deficits observed in unilaterally lesioned rats. These results support the hypothesis that the sole diffusion of DA may improve locomotion and posture deficits observed in Parkinson's disease. Nevertheless, the unilateral lesion model in the rat must be interpreted with caution, and a more stringent model such as the MPTP monkey paradigm, will be necessary to ascertain the clinical potential of this approach.

The present study suggests that polymer capsules loaded with cells secreting large amounts of dopamine may alleviate some of the symptoms of Parkinson's disease. PC12 cells have been reported to secrete large amounts of dopamine, but do form lethal tumors when implanted in the brain (17). When encapsulated in the same polymeric membrane as used for the dopamine rod receptacle, these cells survived for up to 6 months and their growth was constrained to the polymer capsule space (18). Recently, the grafting of genetically engineered fibroblasts which produce L-dopa has been proposed as a source of dopamine (14, 28). Even though these cells are not tumoral, human application may require a way to easily retrieve the transplanted cells such as encasing them in a polymer capsule.

We conclude that a permanent permselective receptacle permits atraumatic placement of polymer rods releasing biologically active molecules into the brain and provides a model for isolating and determining the role of potential factors involved in the reversal of various neurological dysfunctions.

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